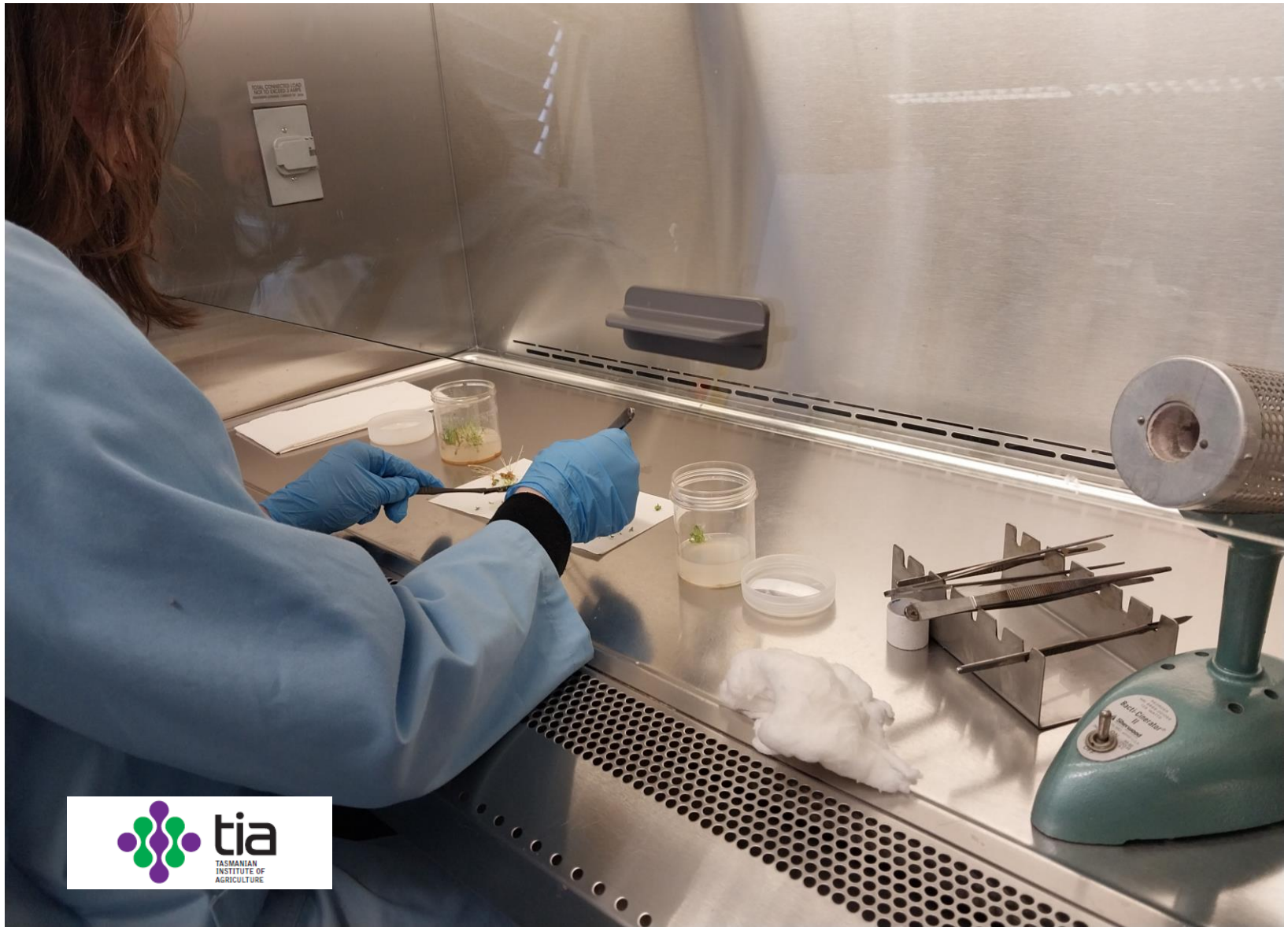
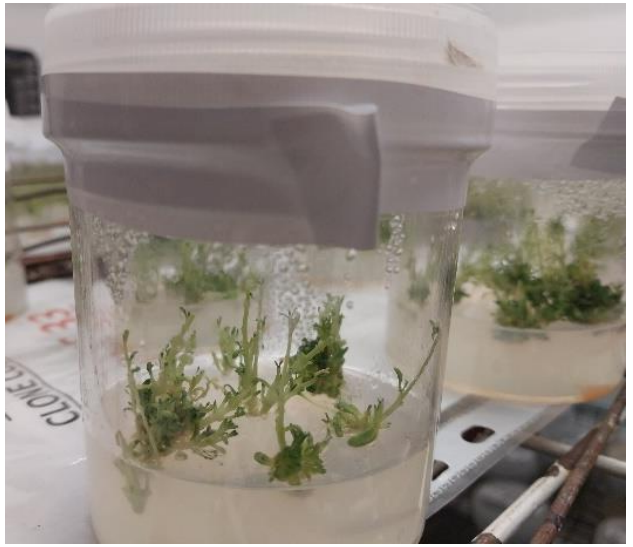
Tissue Culture – *Boronia* case study example





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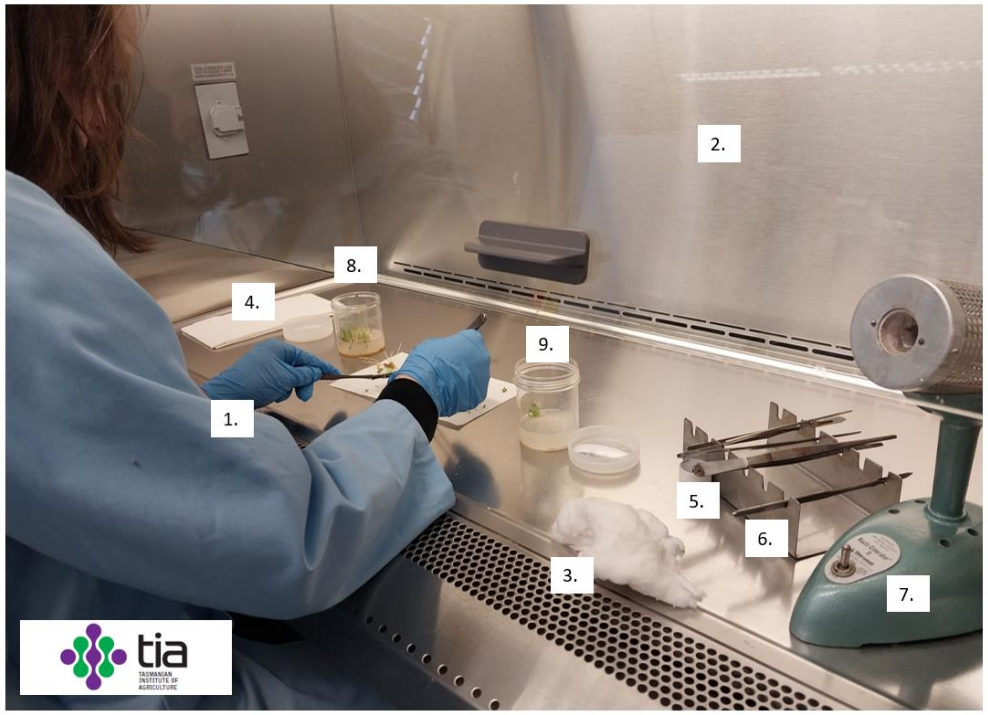
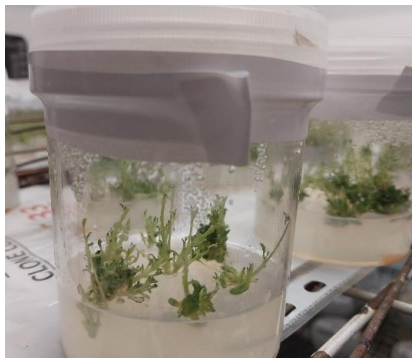
Preparation Steps

- The **laboratory workspace** is set up to include (see photo of student above):
  - (1) **Personal protective equipment (PPE)**: safety equipment is worn when in the laboratory, including – laboratory coat, gloves, closed toed shoes.
  - (2) **a work station** in a ventilated laboratory space is set up to perform the dividing process.
  - (3) **cleaning** of the work surface environment is sterilised (cleaned with ethanol & cotton wool) to reduce the chance of microorganisms contaminating & colonising the samples.
  - **Various laboratory equipment** for the dividing process: sterile paper towel/sheets that are laid in front of the student to divide the plants upon (4); tweezers or forceps for handling the callus/plantlets (5); scalpel for cutting the plant material (6).
  - (7) **A hot sterilising tube** (hot tube that will sterilise equipment when inserted for short periods – e.g., scalpel and tweezers. A flame (Bunsen burner etc.) can alternatively be used for this process.

- **The student collects a jar of *Boronia*** (8) from which multiple developed *Boronia* callus are maturing, containing stems and leaves. This is the organism that will be divided to form new plantlets in a fresh container.

- **A freshly sterilised jar** (9) that has been prepared with the appropriate nutrient agar medium is also placed in the workspace.





Dividing plantlets of *Boronia*:

- The student firstly **sterilises the tweezers and scalpel** in the hot tube (cleans this from any lingering microorganisms that can spoil the experiment).
- Working from left to right, the student gently removes an established *Boronia* plantlet (8) using tweezers, then places this on paper towel.
- They carefully divide the plantlets at the callus, ideally including some callus cell bundles in the next plant, whilst leaving some remaining.
- The new plantlet is placed in the new sterile jar of agar medium (9), placing this on the surface, contacting the gel for its nutrients.
- Continue this process of dividing the same individual (steps 3-4). Once complete, the jar is closed & sealed.
- The scalpels/forceps are sterilised with the hot tube and placed on the rack.
- This process is continued with a fresh batch & repeated.
- All individuals are returned to the controlled environment (refrigerator storage), to be stored for weeks, months or even years until needed or new nutrients are to be provided.
- The student has successfully created tissue culture clones!

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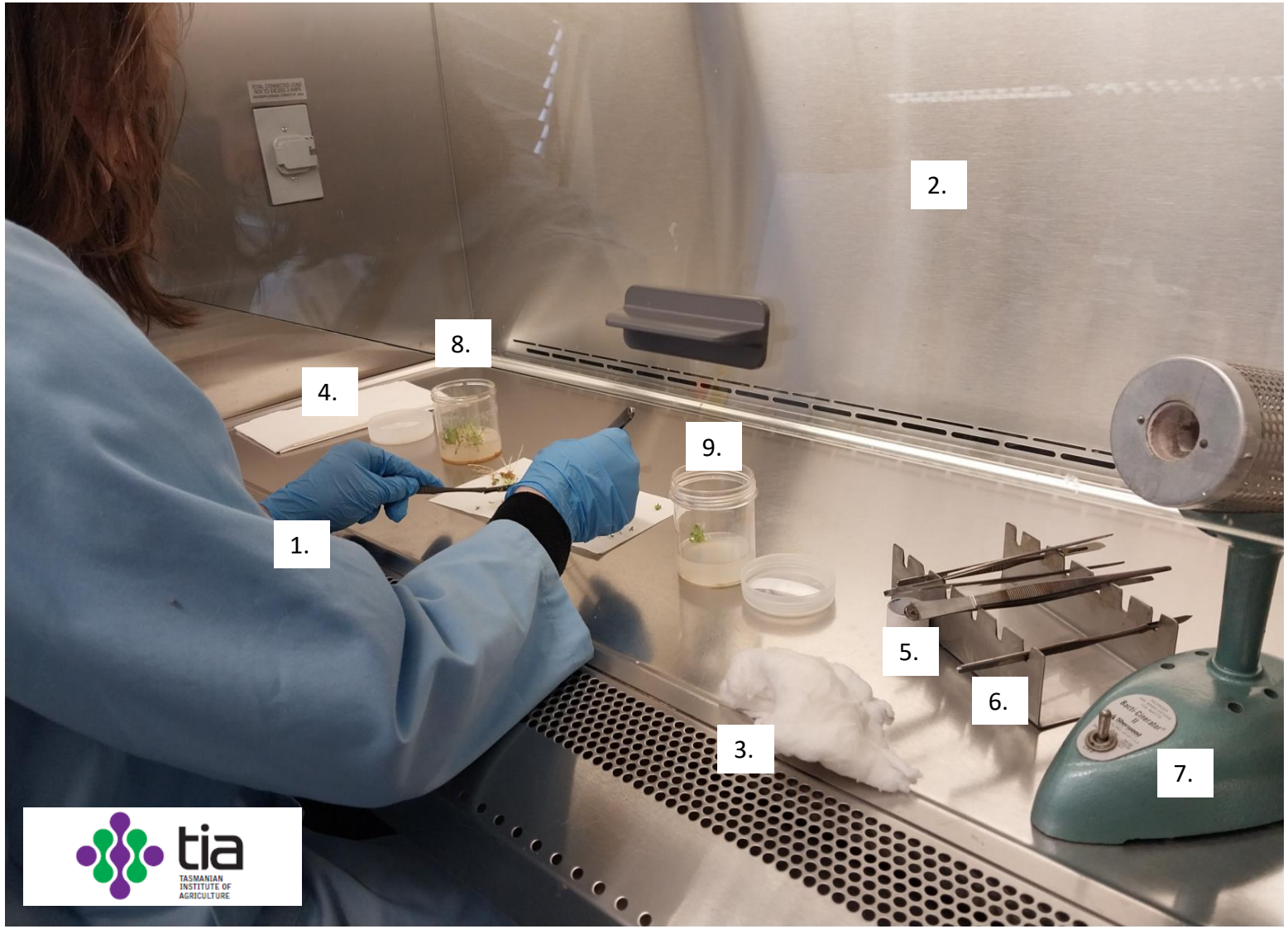
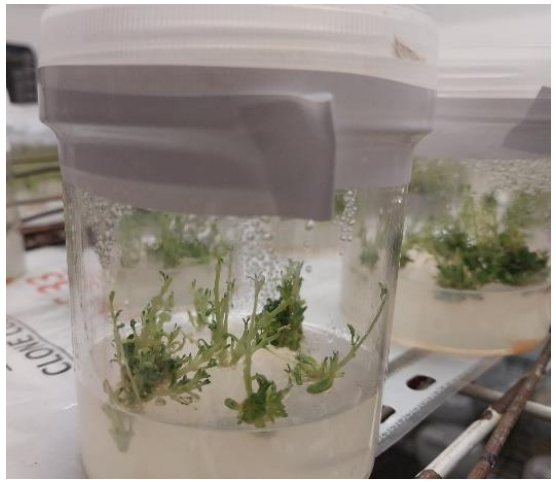
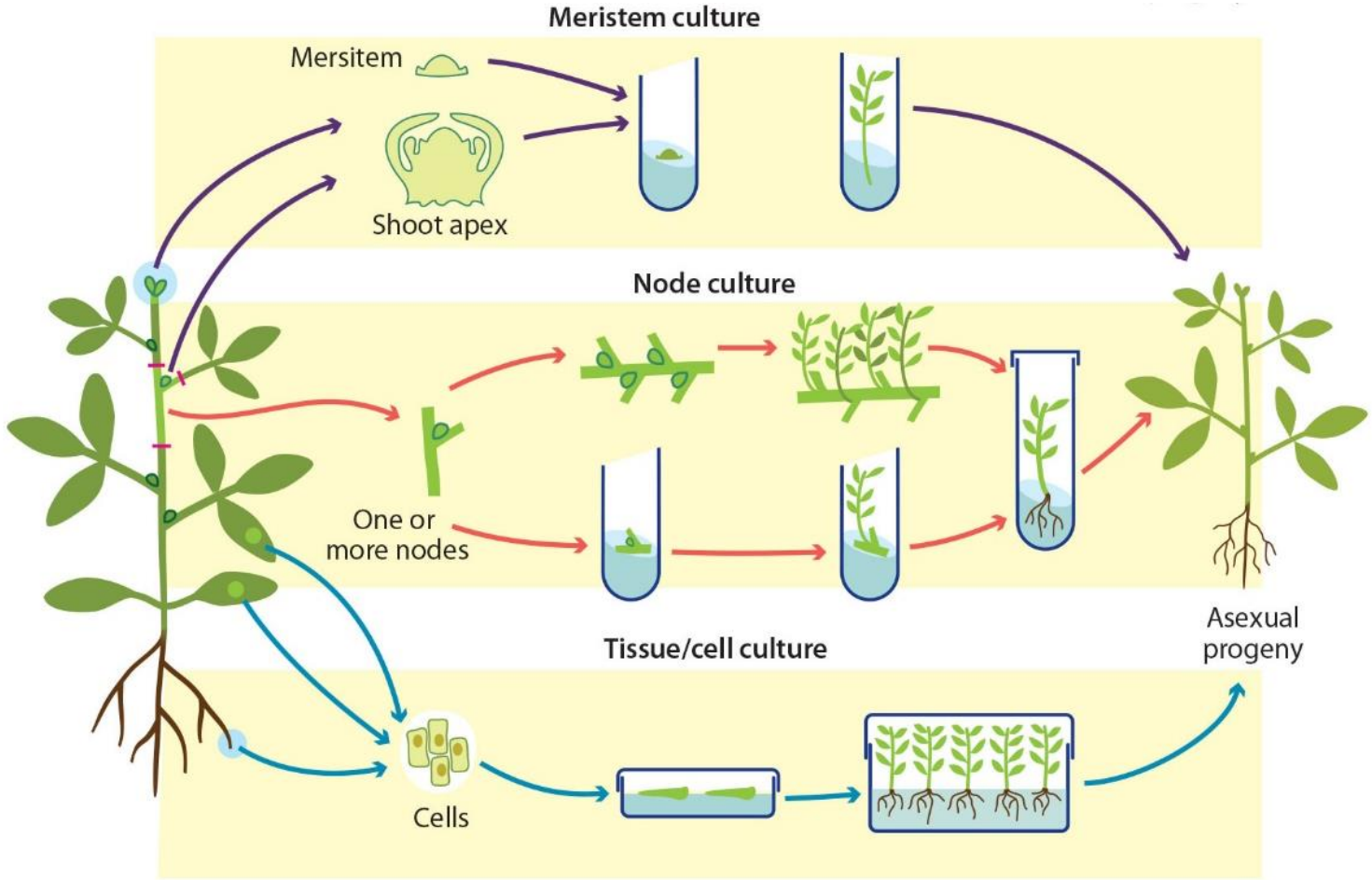
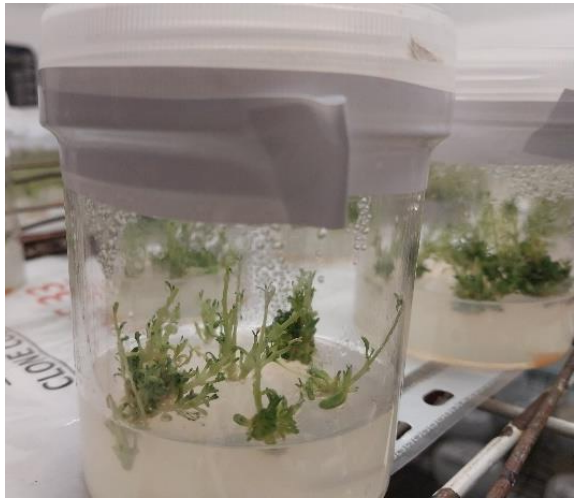




Additional diagram (larger format)





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